ANNALS OF SURGERY
Vol. 228, No. 4, 518–527

Gut-Derived Mesenteric Lymph but not Portal Blood Increases Endothelial Cell Permeability and Promotes Lung Injury After Hemorrhagic Shock

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Objective

To determine whether gut-derived factors leading to organ injury and increased endothelial cell permeability would be present in the mesenteric lymph at higher levels than in the portal blood of rats subjected to hemorrhagic shock. This hypothesis was tested by examining the effect of portal blood plasma and mesenteric lymph on endothelial cell monolayers and the interruption of mesenteric lymph flow on shock-induced lung injury.

Summary Background Data

The absence of detectable bacteremia or endotoxemia in the portal blood of trauma victims casts doubt on the role of the gut in the generation of multiple organ failure. Nevertheless, previous experimental work has clearly documented the connection between shock and gut injury as well as the concept of gut-induced sepsis and distant organ failure. One explanation for this apparent paradox would be that gut-derived inflammatory factors are reaching the lung and systemic circulation via the gut lymphatics rather than the portal circulation.

Methods

Human umbilical vein endothelial cell monolayers, grown in two-compartment systems, were exposed to media, sham-shock, or postshock portal blood plasma or lymph, and permeability to rhodamine (10K) was measured. Sprague–Dawley rats were subjected to 90 minutes of sham or actual shock and shock plus lymphatic division (before and after shock). Lung permeability, pulmonary myeloperoxidase levels, alveolar apoptosis, and bronchoalveolar fluid protein content were used to quantitate lung injury.

Results

Postshock lymph increased endothelial cell monolayer permeability but not postshock plasma, sham-shock lymph/plasma, or medium. Lymphatic division before hemorrhagic shock prevented shock-induced increases in lung permeability to Evans blue dye and alveolar apoptosis and reduced pulmonary MPO levels. In contrast, division of the mesenteric lymphatics at the end of the shock period but before reperfusion ameliorated but failed to prevent increased lung permeability, alveolar apoptosis, and MPO accumulation.

Conclusions

Gut barrier failure after hemorrhagic shock may be involved in the pathogenesis of shock-induced distant organ injury via gut-derived factors carried in the mesenteric lymph rather than the portal circulation.

Gut barrier failure, with the ensuing translocation of bacteria and endotoxin from the gut, has been proposed as a major contributor to the development of systemic infection and multiple organ failure (MOF) after shock.[1] Evidence of the association between gut injury and the subsequent development of a septic state and distant organ failure[2–4] continues to increase. There are abundant clinical and experimental data that trauma and hemorrhage are associated with elevated systemic cytokine levels.[5–7] In fact, MOF, including acute lung injury, after hemorrhagic shock appears to result from an overwhelming systemic inflammatory response driven by the release, activation, and interaction of endogenous cytokines.[8,9] Thus, gut-derived cy-

Presented at the 118th Annual Meeting of the American Surgical Association, April 3, 1998, Palm Beach, Florida.
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Accepted for publication April 23, 1998.

tokinemia may serve as a signal that triggers, perpetuates, and exacerbates the hypermetabolic and immunoinflammatory responses observed after hemorrhagic shock. Many animal[10–12] and human studies have documented that intestinal permeability is increased in a number of circumstances, including shortly after thermal injury,[13] in healthy volunteers receiving a single dose of LPS (lipopolysaccharide) ,[14] and in trauma victims who arrive at the emergency room in shock.[15] However, recent clinical studies in victims of major trauma[16,17] have questioned the clinical relevance of gut-origin sepsis and the role of the gut as an organ responsible for the development of MOF. In these studies, researchers failed to document the presence of detectable bacteria in either the portal blood[16] or the mesenteric lymph nodes[17] of trauma victims, including several patients in whom systemic infections or MOF subsequently developed. One possible explanation for this apparent controversy is that gut ischemia can induce gut-associated lymphoid tissue (GALT) to produce and release cytokines, even in the absence of detectable portal bacteremia—that is, gut-origin sepsis may be mediated by factors carried in the mesenteric lymph rather than the portal blood.[18–20] Also, because the lung is the first vascular bed exposed to mesenteric lymph, shock-induced lung injury may be secondary to either the toxic properties of mesenteric lymph acting directly on endothelial cells or lymph-mediated endothelial cell–neutrophil interactions.

In this context, then, we tested the hypothesis that mesenteric lymph generated after hemorrhagic shock will increase endothelial cell permeability and contribute to lung injury. This hypothesis was tested by examining the effect of mesenteric lymph and portal blood on endothelial cell monolayer permeability and by determining whether interruption of mesenteric lymph flow reduces shock-induced lung injury.

MATERIALS AND METHODS

Animals

Specific pathogen-free male Sprague–Dawley rats (Taconic Farms, Germantown, NY) weighing 350 to 450 g were housed under barrier-sustained conditions and kept at 25°C with 12-hour light/dark cycles. The rats had free access to water and chow (Teklad 22/5 Rodent Diet W-8640, Harlan Teklad, Madison, WI). All rats were maintained in accordance with the recommendations of the "Guide for the Care and Use of Laboratory Animals," and the experiments were approved by the New Jersey Medical School Animal Care Committee.

Human Umbilical Vein Endothelial Cell Permeability

Human umbilical vein endothelial cell (HUVEC; Clonetics, San Diego, CA) monolayers were exposed to lymph and portal blood plasma, and the permeability of the monolayers was assessed by measuring the movement of a rhodamine (10K) probe across the monolayer as follows. HUVECs were grown in completed media supplied by Clonetics according to the company recommendations in a 95% air, 5% CO_2 incubator at 37°C. All HUVEC experiments used cells between the third and tenth passage.

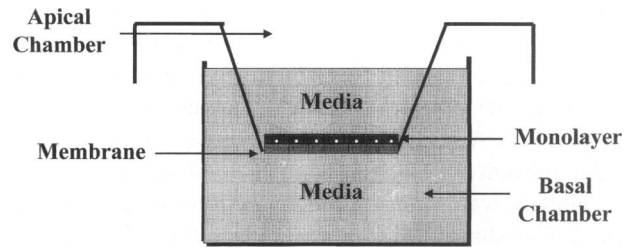

Figure 1. HUVEC permeability assay using the Costar transwell system. After monolayer confluence is achieved, medium alone or medium containing sham-shock portal vein plasma, postshock portal vein plasma, sham-shock lymph, or postshock lymph is placed in the apical chamber and incubated for 1 hour. A 0.05% solution of 10K dextran rhodamine is then added to the apical chamber. After 5 hours of combined incubation, the amount of rhodamine present in the basal chamber is determined spectrophotometrically.

HUVECs were seeded at 20,000 cells per insert (area = 0.33 cm^2) on type I rat-tail collagen-coated membranes (pore size, 3 μm) contained on the apical chamber of a transwell system (Costar, Cambridge, MA) (Fig. 1). When the monolayers had become confluent (72 hours after seeding), they were incubated with media (n = 4), sham-shock portal vein plasma (n = 4, diluted 1:10), postshock portal vein plasma (n = 4, diluted 1:10), sham-shock lymph (n = 4, diluted 1:10), or postshock lymph (n = 4, diluted 1:10) for 1 hour. At this point, a 0.05% solution of 10K dextran rhodamine (Molecular Probe, Eugene, OR) was added to the apical chamber of the transwell system. After 5 hours of combined incubation (plasma or lymph plus probe), the medium in the basal chamber was removed and the amount of dextran rhodamine present was determined spectrophotometrically at 570 nm.

Shock Model

The rats were anesthetized with pentobarbital (50 mg/kg) and the femoral artery was isolated using aseptic techniques and cannulated with polyethylene (PE-50) tubing (0.023 ID, 0.038 OD) containing 0.1 ml of heparinized saline (10 units/cc).[21] The catheter was attached in line to a blood-pressure recorder (Grass Model 79E Data Recorder, Quincy, MA), which was used to monitor the animals' blood pressure continuously during the shock period. Next, aseptic cannulation of the internal jugular vein was performed using a 50-gauge silicone catheter. Blood was withdrawn from the internal jugular vein into a syringe containing 10 units of heparin suspended in 0.3 ml of 0.9% normal saline solution to prevent clotting. The mean arterial pres-

sure was reduced to 30 mmHg and maintained at this level for 90 minutes by withdrawing or reinfusing shed blood (kept at 37°C) as needed. Rectal temperature was monitored throughout the shock period and maintained at approximately 37°C. A heat lamp positioned over the animal was used to prevent hypothermia. At the end of the shock period, the animals were resuscitated by reinfusing all the shed blood. The sham-shock rats were anesthetized and their femoral arteries were cannulated, but no blood was withdrawn or infused.[22]

Division of Mesenteric Lymphatics

A midline celiotomy incision was made and the efferent mesenteric lymphatic vessel identified (adjacent to the superior mesenteric artery) by reflecting the loops of intestine to the left of the animal with moist gauze swabs. Next, the lymphatics were divided with visible confirmation of free flow of lymph into the peritoneal cavity. A sterile gauze was placed in the peritoneal cavity to collect the lymph. At this point, the celiotomy incision was closed and the animal subjected to sham or actual shock. In one group, lymphatic division was performed after the sham or actual shock period. Regardless of the timing of lymphatic division, all animals underwent a 3-hour recovery/reperfusion period.

Lung Permeability

After a 3-hour recovery period, all animals were injected with 10 mg of Evans blue dye through the internal jugular catheter. After 5 minutes (allowing complete circulation of the dye), a blood sample (1.5 ml) was withdrawn from the femoral artery catheter. This blood sample was centrifuged at 1500 rpm at 4°C for 20 minutes, and the resultant plasma was serially diluted to form a standard curve. Twenty minutes after injection of the dye, all rats were killed and the lungs harvested. Bronchoalveolar lavage was performed on the excised lungs using normal saline. Five milliliters of normal saline was instilled, rinsed in and out three times, and collected. The lavage was repeated three times. The combined recovered bronchoalveolar fluid (BALF) was consistently 12.5 ml or greater. The BALF was then centrifuged at 1500 rpm at 4°C for 20 minutes to remove any cells, and the supernatant fluid was assayed spectrophotometrically at 620 nm for dye concentration.[23] The concentration of Evans blue dye in the BALF was then plotted on the standard curve, and the percentage relative to that present in the plasma was determined.

Quantitation of Alveolar Apoptosis

After the bronchoalveolar lavage, one lung was frozen at −70°C until the myeloperoxidase (MPO) assay could be performed, and the second lung was fixed in 10% formalin, processed, and embedded in paraffin. Four-micrometer sections were cut and stained for apoptosis using the TUNEL method. The TUNEL assay was performed using the TACS *In Situ* Apoptosis Detection Kit (Trevigen, Gaithersburg, MD). In brief, tissues were dewaxed and rehydrated and then incubated with Proteinase K for 12 minutes at room temperature. The slides were then treated with 3% hydrogen peroxide for 5 minutes at room temperature to inactivate endogenous peroxidase, rinsed with labeling buffer, covered with Klenow plus dNTP (nucleotide mix), and incubated in a humidified chamber at 37°C for 1 hour. The reaction was terminated by transferring the slides to Stop Buffer solution (at room temperature) for 5 minutes. After washing the slides with phosphate-buffered saline, they were covered with streptavidin–horseradish peroxidase detection solution and incubated in a humidified chamber at room temperature for 20 minutes. Subsequently, the slides were rinsed with phosphate-buffered saline and developed with diaminobenzidene (Stable DAB, Research Genetics, Huntsville, AL) for 8 minutes at room temperature, then rinsed and counterstained with methyl green. Apoptotic cells in 20 different high-power fields were counted by a single blinded observer using light microscopy at 400× magnification. The morphologic characteristics of the apoptotic cells included chromatin condensation, separation of the cell from surrounding cells, cell shrinkage, and formation of apoptotic bodies.[24]

Myeloperoxidase Assay

The previously frozen lung tissue was homogenized for 30 seconds in 4 ml of 20 mM potassium phosphate buffer, pH 7.4, and centrifuged for 30 minutes at 40,000g at 4°C. The pellet was resuspended in 4 ml of 50 mM potassium phosphate buffer, pH 6, containing 0.5 g/dl hexadecyltrimethyl ammonium bromide. Samples were sonicated for 90 seconds at full power, incubated in a 60°C water bath for 2 hours, and centrifuged. One tenth of a milliliter of the supernatant was added to 2.9 ml of 50 mM potassium phosphate buffer, pH 6, containing 0.167 mg/ml *O*-dianisidine and 0.0005% hydrogen peroxide. Absorbance of 460 nm of visible light (A_{460}) was measured for 3 minutes. MPO activity per gram of lung tissue was calculated as follows: MPO activity (units/g tissue) = ($\delta A_{460} \times 13.5$)/lung weight (g), where δA_{460} equals the rate of change in absorbance at 460 nm between 1 and 3 minutes. The coefficient 13.5 was empirically determined such that 1 unit of MPO activity is the amount of enzyme that will reduce 1 μmole peroxide per minute.[25]

Bronchoalveolar Fluid and Plasma Protein Assay

The total protein content (g/dl) of both the BALF and the plasma (sham-shock, hemorrhagic shock, lymphatic division immediately before hemorrhagic shock, and hemorrhagic shock followed by lymphatic division) was determined using a hand refractometer (Milton Roy, Rochester, NY).

Experimental Design

The first set of experiments were performed to assess the effects of mesenteric lymph and portal vein plasma from shocked and sham-shocked rats on endothelial cell permeability using 10K rhodamine red as the permeability probe. Mesenteric lymph was obtained by cannulating the efferent mesenteric lymphatic channel that exits the mesenteric lymph node complex, as previously described.[3] In this experiment, HUVEC monolayers, grown in two-compartment systems, were exposed to medium, mesenteric lymph, or portal blood plasma (diluted 1:10) obtained from rats 6 hours after the induction of an episode of sham-shock or hemorrhagic shock (30 mmHg for 90 minutes). Endothelial cell permeability was quantitated by measuring the amount of rhodamine red that crossed the monolayer.

The second group of experiments tested whether division of the mesenteric lymphatics, either before or immediately after an episode of hemorrhagic shock, would prevent or reduce shock-induced pulmonary injury. Rats, anesthetized with 50 mg/kg of pentobarbital sodium given intraperitoneally, were placed into one of the following four groups: sham shock (n = 8), hemorrhagic shock (n = 8, mean arterial pressure 30 mmHg), lymphatic division immediately before hemorrhagic shock (n = 8), or hemorrhagic shock followed by lymphatic division (n = 8). After a 3-hour recovery period, all animals received an intravenous injection of 10 mg of Evans blue dye. Twenty minutes later, the rats were killed, the lungs were harvested, bronchoalveolar lavage was performed, and BALF was analyzed spectrophotometrically. The lungs were then fixed in formalin, processed, and embedded in paraffin. Sections of 4 μm were cut and stained for apoptosis. Representative samples from each group were then examined for apoptosis by counting the number of apoptotic nuclei in 20 high-power fields. MPO activity was used to quantitate neutrophil sequestration in the lung.

Statistical Analysis

All data were analyzed by analysis of variance with the Tukey–Kramer multiple comparisons test. All data are expressed as the mean ± standard deviation. Statistical significance was considered to be reached at $p \leq 0.05$.

RESULTS

The permeability of the endothelial cell monolayers to the 10K rhodamine permeability probe was increased to the greatest extent in the monolayers incubated with postshock mesenteric lymph (Fig. 2). The postshock portal vein plasma produced a small but significant increase in monolayer permeability, but the effect was much less than that caused by postshock lymph. These results indicate that postshock lymph has a greater effect on endothelial permeability than does postshock portal vein plasma.

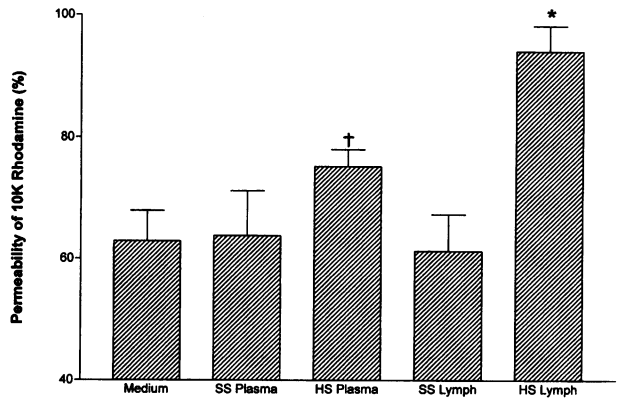

Figure 2. Comparison of the effects of sham-shock (SS) and postshock (HS) portal vein plasma and mesenteric lymph on the permeability of endothelial cell monolayers (n = 4 per group). Data are expressed as mean percentage ± SD of rhodamine that crossed the HUVEC monolayer. *$p < 0.01$ *vs.* all other groups; †$p < 0.05$ *vs.* medium and SS lymph.

Lung permeability to Evans blue dye was greatest in the rats subjected to 90 minutes of hemorrhagic shock (Table 1). Division of the mesenteric lymphatics before the hemorrhagic shock episode completely prevented this increase in pulmonary permeability. In contrast, lymphatic division after the shock period but before reperfusion only partially prevented the shock-induced increase in lung permeability. There was no statistical difference in pulmonary permeability between the shocked rats whose lymphatics were divided before the shock episode and the sham-shocked animals.

Because MPO levels are a reliable and quantitative marker for neutrophil accumulation in tissues,[26] pulmonary MPO levels were used to assess the degree of pulmonary neutrophil infiltration. Lung MPO levels were significantly higher in the shocked rats than in the sham-shocked rats (Table 2). Preshock lymphatic division reduced pulmonary MPO accumulation to a greater extent than postshock lymphatic division. Although both preshock and postshock lymphatic division reduced pulmonary neutrophil sequestration, neither fully prevented the shock-induced increases in lung MPO (see Table 2).

Table 1. EFFECT OF PRE- AND POST SHOCK LYMPHATIC DIVISION (LD) ON LUNG PERMEABILITY

Group	n	% Evans Blue Dye in BALF
Sham shock	8	3.3 ± 0.9
Hemorrhagic shock	8	10.1 ± 1.9*
LD + shock	8	3.4 ± 1.0
Shock + LD	8	7.2 ± 0.8†

Data are expressed as mean ± S.D. Statistical analysis was via ANOVA with Tukey-Kramer Multiple Comparisons Test.
* $p < 0.001$ *vs.* all groups.
† $p < 0.001$ *vs.* sham shock and LD + shock group.

Table 2. EFFECT OF PRE- AND POST SHOCK LYMPHATIC DIVISION (LD) ON PULMONARY MYELOPEROXIDASE ACTIVITY

Group	n	MPO Activity (Units/gm tissue)
Sham shock	8	10.0 ± 1.6
Hemorrhagic shock	8	20.4 ± 2.4*
LD + shock	8	13.3 ± 0.9†
Shock + LD	8	15.8 ± 1.6‡

Data are expressed as mean ± S.D. Statistical analysis was via ANOVA with Tukey-Kramer Multiple Comparisons Test.
* $p < 0.001$ *vs.* all groups.
† $p < 0.01$ *vs.* sham shock group.
‡ $p < 0.001$ *vs.* sham shock and < 0.05 *vs.* LD + shock group.

Table 4. EFFECT OF PRE- AND POST SHOCK LYMPHATIC DIVISION (LD) ON BALF PROTEIN CONTENT AND BALF/PLASMA PROTEIN RATIO

Group	n	BALF Protein (gm/dl)	BALF/Plasma Protein Ratio
Sham shock	8	1.3 ± 0.2	0.15 ± 0.02
Hemorrhagic shock	8	2.1 ± 0.1*	0.36 ± 0.03*
LD + shock	8	1.3 ± 0.1	0.16 ± 0.01
Shock + LD	8	1.6 ± 0.2†	0.24 ± 0.02†

BALF = bronchoalveolar fluid
Data are expressed as mean ± S.D. Statistical analysis was via ANOVA with Tukey-Kramer Multiple Comparisons Test.
* $p < 0.001$ *vs.* all groups.
† $p < 0.001$ *vs.* sham shock and < 0.01 *vs.* LD + shock group.
‡ $p < 0.001$ *vs.* sham shock and LD + shock group.

The incidence of alveolar apoptosis increased significantly in the rats subjected to 90 minutes of hemorrhagic shock *versus* both sham-shocked and shocked animals whose mesenteric lymphatics had been divided before the shock period (Table 3). Lymphatic division before the shock period completely prevented shock-induced alveolar apoptosis, whereas lymphatic division after the shock period only partially reduced this increase in shock-induced alveolar apoptosis.

After hemorrhagic shock, there was a significant increase in both the total protein content of the BALF as well as the BALF/plasma protein ratio (Table 4). Division of the mesenteric lymphatics before the shock episode completely prevented the shock-induced increase in BALF protein content. As was true of lung permeability as measured by Evans blue dye, postshock lymphatic division reduced but failed to prevent the shock-induced increase in the BALF protein content.

DISCUSSION

Although there is abundant clinical and experimental evidence documenting the connection among shock, gut injury, and the subsequent development of distant organ failure,[1,27,28] the possibility that gut-derived cell-injurious factors are reaching the lung and systemic circulation via the intestinal lymphatics has not been tested to any degree. Not only does the gut-associated lymphoid tissue make up the body's largest lymphoid "organ," but the intestinal immune system is also continually exposed to potential proinflammatory stimuli.[29] Because hemorrhagic shock is associated with intestinal ischemia, loss of gut barrier function, and subsequent bacterial translocation, it appears logical that the level of GALT activation should also be increased. In fact, our results support the hypothesis that after a nonlethal but severe episode of hemorrhagic shock, gut-derived factors are present in the mesenteric lymph to a much higher extent than in the portal circulation, and that these factors contribute to increased endothelial cell permeability and shock-induced lung injury. This conclusion is based on the following experimental observations. First, the *in vitro* studies demonstrated that postshock mesenteric lymph increased the passage of the 10K rhodamine permeability probe across HUVEC monolayers. Second, lymphatic division either completely prevented or reduced (depending on the timing of division) the shock-induced increases observed in lung permeability, MPO accumulation, alveolar apoptosis, and BALF protein content.

Hemorrhagic shock resulted in a significant and reproducible increase in lung permeability, as measured by Evans blue dye extravasation into the lung, that was approximately threefold higher than that observed in the sham-shocked animals. Lymphatic division before induction of the shock state prevented this increase in lung permeability. Preshock lymphatic division also prevented the increase in the incidence of shock-induced alveolar apoptosis and the amount of BALF protein. These complementary findings support the concept that gut-derived lymph has a potential role in the generation of postshock lung injury. One explanation for this observation may be that during the shock period, the ischemic gut produces or contains factors that, because of

Table 3. EFFECT OF PRE- AND POST SHOCK LYMPHATIC DIVISION (LD) ON ALVEOLAR APOPTOSIS

Group	HPF	# Apoptotic Nuclei/HPF
Sham shock	80	1.1 ± 1.0
Hemorrhagic shock	80	4.6 ± 2.3*
LD + shock	80	1.0 ± 0.9
Shock + LD	80	2.6 ± 0.9†

HPF = high power field.
Data are expressed as mean ± S.D. Statistical analysis was via ANOVA with Tukey-Kramer Multiple Comparisons Test.
* $p < 0.001$ *vs.* all groups.
† $p < 0.001$ *vs.* sham shock and LD + shock group.

the loss of gut barrier function, are preferentially transported via the lymphatic circulation. These factors are then carried via the lymphatic route to the lung, which represents the first vascular bed to be exposed to the mesenteric lymph. This process then leads to an increase in alveolar apoptosis, which in turn results in increased protein leak and lung permeability. Thus, lymphatic division before hemorrhagic shock prevents the subsequent transport of the factors produced during the period of gut ischemia. In contrast, division of the mesenteric lymphatics at the end of the shock period but before reperfusion reduces but does not prevent these shock-induced changes. This is most likely secondary to the fact that most of the factors that increase lung injury are released during the shock period itself, with only a small but measurable amount being released during the reperfusion period.

The observation that pulmonary MPO activity increased after hemorrhagic shock is consistent with similar models of gut ischemia/reperfusion.[18,27,30] Interestingly, lymphatic division, both before and after shock, failed to prevent the observed increase in MPO accumulation seen with hemorrhagic shock. One possible explanation for this observation may be that after mesenteric reperfusion, circulating neutrophils are primed either by direct interaction with the endothelium of the injured gut or by inflammatory mediators released from the gut[30] into the mesenteric lymphatics or the portal circulation. Hence, the lymph is only partially responsible for the activation and recruitment of pulmonary neutrophils, thereby accounting for the greater decrease in MPO accumulation seen with preshock lymphatic division than postshock division.

The question then becomes what factors are in the lymph, and how do they contribute to shock-induced lung injury. Koike et al.[31] demonstrated that mesenteric ischemia activated intestinal phospholipase A_2, resulting in neutrophil priming and subsequent lung albumin leak. Rats, subjected to 45 minutes of intestinal ischemia and 6 hours of reperfusion, showed evidence of phospholipase A_2 activation during the ischemic insult and circulating neutrophil priming 1 hour after reperfusion. Exaggerated albumin lung leak occurred after 2 hours of reperfusion, whereas treatment with a phospholipase A_2 inhibitor within 15 minutes of reperfusion diminished subsequent neutrophil priming and lung leak. In another model,[32] mesenteric lymph produced after hemorrhagic shock was found to contain elevated levels of both interleukin-6 and tumor necrosis factor. In fact, when compared with both portal and systemic blood, lymphatic cytokine levels were consistently higher, especially in the rats subjected to the longest periods of hemorrhagic shock. Finally, Olofsson et al.,[33] using a partial gut ischemia model in rats, demonstrated that the development of systemic endotoxemia paralleled the appearance of endotoxin in thoracic duct lymph, and endotoxin was identified in the lymph before significant portal endotoxemia.

Consequently, from the results of the present study, one possible and plausible mechanistic sequence from gut injury (ischemia/reperfusion) after hemorrhagic shock to lung injury involves:

1. Elaboration of proinflammatory mediators from the gut
2. Subsequent transport of these mediators into the lung via the intestinal lymphatics
3. Induction of alveolar apoptosis
4. Lung injury.

CONCLUSIONS

The concept that the lymphatic route is the major route by which gut-derived factors reach the systemic circulation is supported by several lines of evidence. First, we know from many animal studies that the primary route of bacterial translocation is via the mesenteric lymph, and that the mesenteric lymph is the first and frequently the only tissue to contain translocated bacteria.[32] Second, multiple different experimental models,[33–35] including a model of gut ischemia,[33] reveal significantly elevated levels of gut-derived endotoxin in the thoracic duct before its subsequent detection in the portal circulation. Finally, studies involving trauma patients demonstrate increased evidence of bacterial translocation in culture-negative mesenteric lymph nodes through the detection of β-galactosidase[36] and electron microscopic evaluation of the mesenteric lymph node.[37] Thus, intestinal bacteria do not need to reach the portal circulation to induce a systemic inflammatory state. Instead, loss of gut barrier function to bacteria (or endotoxin), especially if coupled with gut injury, could produce a local intestinal inflammatory response and lead to the subsequent liberation of cytokines from the GALT. Further, because the biologic effects of bacteria and endotoxin are mediated primarily by host-derived responses, bacterial translocation may be involved in the pathogenesis of the systemic inflammatory state and distant organ injury via activation of the GALT, despite the absence of detectable portal bacteria. If this is the case, it would not be necessary to recover bacteria from either the portal or systemic circulation for gut injury to contribute to a systemic inflammatory state and distant organ injury.

The present study was carried out to test whether hemorrhagic shock can generate mesenteric lymph that increases tissue permeability and results in organ (lung) injury. The results of this study, documenting that postshock mesenteric lymph increases endothelial cell monolayer permeability and that division of mesenteric lymph flow prevents shock-induced lung injury, support this hypothesis. Further studies will be required to elucidate the causative factor carried in the mesenteric lymph.

References

1. Deitch EA. Multiple organ failure pathophysiology and potential future therapy. Ann Surg 1992; 216:117–134.
2. Johnston TD, Fischer R, Chen Y, Reed RL. Lung injury from gut ischemia: insensitivity to portal blood flow diversion. J Trauma 1993; 5:508–511.

3. Deitch EA, Xu DZ, Franko L, Ayala A, Chaudry IH. Evidence favoring the role of the gut as a cytokine-generating organ in rats subjected to hemorrhagic shock. Shock 1994; 1:141–146.
4. Mainous MR, Ertel W, Chaudry IH, Deitch EA. The gut: a cytokine-generating organ in systemic inflammation? Shock 1995; 4:193–199.
5. Ayala A, Wang P, Ba ZF, Perrin, Ertel W, Chaudry IH. Differential alterations in plasma IL-6 and TNF levels after trauma and hemorrhage. Am J Physiol 1991; 260:R167–R171.
6. Endo S, Inada K, Yamada Y, et al. Plasma endotoxin and cytokine concentrations in patients with hemorrhagic shock. Crit Care Med 1994; 22:949–955.
7. Roumen RM, Hendriks T, van der Ven Jongekrijg J, et al. Cytokine patterns in patients after major vascular surgery, hemorrhagic shock and severe blunt trauma. Relation with subsequent adult respiratory distress syndrome and multiple organ failure. Ann Surg 1993; 218:769–776.
8. Peitzman AB, Billiar TR, Harbrecht BG, Kelly E, Udekwu AO, Simmons RL. Hemorrhagic shock. Curr Probl Surg 1995; 32:925–1002.
9. Fong Y, Moldawer LL, Shires GT, Lowry SF. The biologic characteristics of cytokines and their implication in surgical injury. Surg Gynecol Obstet 1990; 170:363–378.
10. Maejima K, Deitch EA, Berg RD. Bacterial translocation from the gastrointestinal tracts of rats receiving thermal injury. Infect Immun 1984; 43:6–10.
11. Xu D, Lu Q, Guillory D, Cruz N, Berg R, Deitch EA. Mechanisms of endotoxin-induced intestinal injury in a hyperdynamic model of sepsis. J Trauma 1993; 34:676–682.
12. Deitch EA, Morrison J, Berg R, Specian RD. Effect of hemorrhagic shock on bacterial translocation, intestinal morphology and intestinal permeability in conventional and antibiotic decontaminated rats. Crit Care Med 1990; 18:529–536.
13. Deitch EA. Intestinal permeability is increased in burn patients shortly after injury. Surgery 1990; 107:411–416.
14. O'Dwyer ST, Mitchie HR, Ziegler TR, Revhaug A, Smith RJ, Wilmore DW. A single dose of endotoxin increases intestinal permeability in humans. Arch Surg 1988; 123:1459–1464.
15. Rush BF, Sori AJ, Murphy TF, Smith S, Flanagan JJ, Machiedo GW. Endotoxemia and bacteremia during hemorrhagic shock. The link between trauma and sepsis? Ann Surg 1988; 207:549–554.
16. Moore FA, Moore EE, Poggetti R, et al. Gut bacterial translocation via the portal vein: a clinical perspective with major torso trauma. J Trauma 1991; 31:629–638.
17. Peitzman AB, Udekwu AO, Ochoa J, Smith S. Bacterial translocation in trauma patients. J Trauma 1991; 31:1083–1087.
18. Schmeling DJ, Caty MG, Oldham KT, Guice KS, Hinshaw DB. Evidence for neutrophil-related acute lung injury after intestinal ischemia-reperfusion. Surgery 1989; 106:195–202.
19. Poggetti RS, Moore FA, Moore EE, Koike K, Banerjee A. A simultaneous liver and lung injury following gut ischemia is mediated by xanthine oxidase. J Trauma 1992; 32:723–728.
20. Gerkin TM, Oldham KT, Guice KS, Hinshaw DB, Ryan US. Intestinal ischemia-reperfusion injury causes pulmonary endothelial cell ATP depletion. Ann Surg 1993; 217:48–56.
21. Baker JW, Deitch EA, Ma L, Berg RD, Specian RD. Hemorrhagic shock induces bacterial translocation from the gut. J Trauma 1988; 28:896–906.
22. Deitch EA, Bridges B, Baker J, et al. Hemorrhagic shock-induced bacterial translocation is reduced by xanthine oxidase inhibition or inactivation. Surgery 1988; 104:191–198.
23. Patterson CE, Rhoades RA, Garcia JGN. Evans blue dye as a marker of albumin clearance in cultured endothelial monolayer and isolated lung. J Appl Physiol 1992; 72:865–873.
24. Cohen JJ. Overview: mechanisms of apoptosis. Immunol Today 1993; 14:126–130.
25. Anderson BO, Brown JM, Shanley PF, Benserd DD, Harken AH. Marginating neutrophils are reversibly adherent to normal lung endothelium. Surgery 1991; 109:51–61.
26. Allan G, Bhattacherjee P, Brook CD, Read NG, Parke AJ. Myeloperoxidase activity as a quantitative marker of polymorphonuclear leukocyte accumulation into an experimental myocardial infarct—the effect of ibuprofen on infarct size and polymorphonuclear leukocyte accumulation. J Cardiovasc Pharmacol 1985; 7:1154–1160.
27. Koike K, Moore EE, Moore FA, Read RA, Carl VS, Banerjee A. Gut ischemia/reperfusion produces lung injury independent of endotoxin. Crit Care Med 1994; 22:1438–1444.
28. Koike K, Moore EE, Moore FA, Franciose RJ, Fontes B, Kim FJ. CD11b blockade prevents lung injury despite neutrophil priming after gut ischemia/reperfusion. J Trauma 1995; 39:23–27.
29. Nagura H, Sumi Y. Immunological functions of the gut—role of the mucosal immune system. Toxicol Pathol 1988; 16:154–164.
30. Simpson R, Alon R, Kobzik L, Valeri CR, Shepro D, Hechtman HB. Neutrophil- and nonneutrophil-mediated injury in intestinal ischemia-reperfusion. Ann Surg 1993; 218:444–454.
31. Koike K, Moore EE, Moore FA, Kim FJW, Carl VS, Banerjee A. Gut phospholipase A2 mediates neutrophil priming and lung injury after mesenteric ischemia-reperfusion. Am J Physiol 1995; 268:G397–G403.
32. Deitch EA. Bacterial translocation of the gut flora. J Trauma 1990; 30:184–189.
33. Olofsson P, Nylander G, Olsson P. Endotoxin transport routes and kinetics in intestinal ischemia. Acta Chir Scand 1985; 151:635–639.
34. Olofsson P, Nylander G, Olsson P. Endotoxin routes of transport in experimental peritonitis. Am J Surg 1986; 151:443–446.
35. Gans H, Matsumoto K. The escape of endotoxin from the intestine. Surg Gynecol Obstet 1974; 139:395–402.
36. Braithwaite CEM, Ross SE, Nagele R, Mure AJ, O'Malley KF, Garcia-Perez FA. Bacterial translocation occurs in humans after traumatic injury: evidence using immunofluorescence. J Trauma 1993; 34:586–590.
37. Reed LL, Martin M, Manglano R, Newman B, Kocka F, Barrett J. Bacterial translocation following abdominal trauma in humans. Circ Shock 1994; 42:1–6.

Discussion

DR. PAUL H. JORDAN, JR. (Houston, Texas): Since your experiment is dependent upon the inhibition of lymphatic flow, the audience may be interested to know how this is done in animals so that you know it is complete?

DR. EDWIN A. DEITCH (Newark, New Jersey): There are two ways of doing it. One is to put a cannula in the lymphatic and then drain the lymphatic duct externally. The second way is what we did here, which is to divide the lymphatic duct and let the lymph accumulate within a gauze pad.

DR. PAUL H. JORDAN, JR. (Houston, Texas): So there is one lymphatic in the animals that drains all the lymph from the abdomen.

DR. EDWIN A. DEITCH (Newark, New Jersey): That is right. The intestinal lymph drains through one common lymphatic channel that exits at the confluence of the mesenteric lymph nodes.

DR. PER-OLOF J. HASSELGREN (Cincinnati, Ohio): The study addresses a clinically important question, that is, if and how gut-derived factors can induce lung injury following shock. The authors have convincingly shown that a factor or factors released from the intestine and splanchnic bed following hemorrhagic